Bayer HealthCare

QC

HAV Total (HAVT)

Contents

REF	Contents
01313752	2 vials of Negative Control CONTROL
	2 vials of Positive Control CONTROL
	Expected Values Card and barcode labels

01861393 Rev. A, 2005-02

Intended Use

For monitoring the performance of the HAV Total Assay on ADVIA Centaur® Systems. The performance of the HAV Total quality control material has not been established with any other anti-HAV Total Assay.

"**WARNING**: United States federal law restricts this device to sale by or on the order of a physician."

Control Description

Volume	Ingredients	Storage	Stability
7.0 mL/vial	Recalcified human plasma negative and positive for anti-HAV antibodies with sodium azide (< 0.1%)	2–8°C	Until the expiration date on the vial label or 60 days after opening the vial or onboard–8 hours

WARNINGS:

For *In Vitro* Diagnostic Use.

CAUTION! POTENTIAL BIOHAZARD: The controls contain human source material. No known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. All products manufactured using human source material should be handled as potentially infectious. Handle this product according to established good laboratory practices and universal precautions.[1-3] Use eye protection and gloves when handling this product; wash hands after handling.

The control materials have been assayed by FDA-approved methods and found nonreactive for hepatitis B surface antigen (HBsAg), antibody to hepatitis C (HCV), and antibody to HIV-1/2. The positive control contains human plasma that is reactive for anti-HAV total, but negative for anti-HAV IgM.

NOTE: Sodium azide can react with copper and lead plumbing to form explosive metal azides. On disposal, flush reagents with a large volume of water to prevent the buildup of azides, if disposal into a drain is in compliance with federal, state, and local requirements.

Dispose of hazardous or biologically contaminated materials according to the practices of your institution. Discard all materials in a safe and acceptable manner, and in compliance with all federal, state, and local requirements.

The results obtained using the HAV Total quality control material depend on several factors. Erroneous results can occur from improper storage, inadequate mixing, or other sample handling errors.

The controls are not calibrators and should not be used for assay calibration.

Preparing the Quality Control Material

Gently swirl and invert the vials to ensure homogeneity.

Using the Barcode Labels

NOTE: Control barcode labels are lot number specific. Do not use barcode labels from one lot of controls with any other lot of controls.

Use the HAV Total quality control barcode labels to identify the positive and negative sample cups when performing the ADVIA Centaur HAV Total assay. Place the barcode label on the sample cup so that the readable characters on the side of the label are vertical on the sample cup.

Performing Quality Control

For detailed information about entering quality control values, refer to the system operating instructions or to the online help system.

To monitor system performance and chart trends, as a minimum requirement, quality control samples should be assayed on each workshift that samples are analyzed. Quality control samples should also be assayed when performing a two-point calibration. Treat all quality control samples the same as patient samples.

NOTE: This procedure uses control volumes sufficient to measure each control in duplicate.

1. Schedule the quality control samples to the worklist.
2. Label two sample cups with quality control barcode labels: one for the positive, and another for the negative.

NOTE: Each drop from the control vial is approximately 50 µL.

3. Gently mix the quality control materials and dispense at least 5 to 6 drops into the appropriate sample cups.
4. Load the sample cups in a rack.
5. Place the rack in the sample entry queue.
6. Ensure that the assay reagents are loaded.
7. Start the entry queue, if required.

CAUTION: Dispose of any quality control materials remaining in the sample cups after 8 hours. Do not refill sample cups when the contents are depleted; if required, dispense fresh quality control materials.

Do not return any quality control materials back into the vials after testing because evaporation and contamination can occur, which may affect results.

Reviewing, Editing, and Printing Results

For detailed information about reviewing, editing, and printing quality control results, refer to the system operating instructions or to the online help system.

Expected Results

Refer to the *Expected Values* card for the assigned values specific for the lot number of the HAV Total quality control material. For additional information, refer to the HAV Total assay instructions for use.

The mean values established should fall within the range specified on the *Expected Values* card. Individual results may fall outside the range.

Taking Corrective Action

If the quality control results do not fall within the suggested *Expected Values*, then do the following:

- consider the sample results invalid and repeat testing if controls are out of range
- review these instructions to ensure that the assay was performed according to the procedures recommended by Bayer HealthCare
- verify that the materials are not expired
- verify that required maintenance was performed
- if necessary contact Bayer HealthCare for more assistance

Limitations

Assay values for assays other than the ADVIA Centaur have not been established. If the user wishes to use this control material with other assays, it is their responsibility to establish appropriate ranges.

Technical Assistance

For customer support, please contact your local technical support provider or distributor.

References

1. National Committee for Clinical Laboratory Standards. Procedures for the Handling and Processing of Blood Specimens; Approved guideline-2nd Edition. NCCLS document H18-A2. Wayne (PA):NCCLS;1999
2. Centers for Disease Control. Update: Universal precautions for prevention of transmission of human immunodeficiency virus, hepatitis B virus and other bloodborne pathogens in healthcare settings. MMWR 1988;37:377-82, 387-8.
3. National Committee for Clinical Laboratory Standards. Protection of laboratory workers from instrument biohazards and infectious disease transmitted by blood, body fluids, and tissue; approved guideline. NCCLS Document M29-A2. Wayne (PA):NCCLS;2001.

HAV Total (HAVT)

Assay for the Detection of Total Antibodies to Hepatitis A Virus

Assay Summary

Sample Type	*Serum, potassium EDTA plasma, lithium or sodium heparinized plasma*
Sample Volume	*20 µL*
Calibrator	*HAVT*

Contents

REF	Contents	Number of Tests
07720961	1 ReadyPack® primary reagent pack containing ADVIA Centaur HAVT Lite Reagent, Solid Phase, and Antigen Reagent 1 Ancillary pack containing ADVIA Centaur HAVT Ancillary reagent ANC ADVIA Centaur HAVT Master Curve card 1 vial HAVT Low Calibrator CAL L 1 vial HAVT High Calibrator CAL H ADVIA Centaur HAVT Calibrator Assigned Value card	100

For a definition of symbols used in product labeling, please refer to Appendix D, *Understanding the Symbols,* in the ADVIA Centaur® Assay Manual.

Intended Use

The ADVIA Centaur HAV Total assay is an *in vitro* diagnostic immunoassay for the qualitative determination of total antibodies to hepatitis A virus (anti-HAV) in human serum or plasma (potassium EDTA, lithium or sodium heparinized) using the ADVIA Centaur System. This anti-HAV assay is indicated as an aid in the diagnosis of previous or ongoing hepatitis A viral infection or in the identification of HAV-susceptible individuals for vaccination.

Assay performance characteristics have not been established for immunocompromised or immunosuppressed patients, cord blood, neonatal specimens, infants, or children.

WARNING: This assay has not been FDA cleared or approved for the screening of blood or plasma donors.

United States federal law restricts this device to sale by or on the order of a physician.

Materials Required But Not Provided

REF	Description	Contents
	ADVIA Centaur System	
03395373	ADVIA Centaur Ancillary Probe Wash 1 APW	2 Readypack ancillary reagent packs containing 25 mL per pack
01313752	ADVIA Centaur HAVT quality control material	2 x 7.0 mL Negative Control CONTROL 2 x 7.0 mL Positive Control CONTROL Expected Value card
01137199 (112351)	ADVIA Centaur Wash 1 WASH 1	2 x 1500 mL/pack

Summary and Explanation of the Test

The ADVIA Centaur HAV Total assay is a competitive chemiluminometric immunoassay used for the detection of total antibody to hepatitis A virus in human serum or plasma.

Hepatitis A is caused by infection with the hepatitis A virus. HAV is a 27-nanometer single-stranded, non enveloped, RNA virus that is classified as a picornavirus. Transmission of hepatitis A is via the fecal-oral route, and infection occurs mainly due to contaminated food or poor sanitary conditions.[1,2]

Hepatitis A virus replicates in the liver. The virus is excreted in the bile and shed in the stool. Only one serotype has been observed among HAV isolates collected from various parts of the world. The average incubation period for HAV infection is 30 days with a range of 15 to 40 days. Chronic infection has not been reported to occur following HAV infection. Symptoms last approximately 2 weeks and include hepatomegaly, jaundice, dark urine, fatigue, and gastrointestinal distress such as anorexia, nausea, vomiting, and abdominal pain. At the onset of symptoms resulting from HAV infection, antibody to HAV is detectable. The early antibody response is largely comprised of the IgM antibody subclass. Anti-HAV IgM is detectable for 3 to 6 months after the onset of illness, whereas anti-HAV IgG can persist indefinitely. The specific determination of anti-HAV IgM is the most useful serological marker for diagnosing acute HAV infection. Total anti-HAV is used primarily for determination of previous exposure to Hepatitis A virus.[1-4]

The ADVIA Centaur HAV Total assay detects all classes of antibodies against hepatitis A virus. The measurement of anti-HAV total activity is used to identify HAV susceptible individuals for vaccination.[5,6]

Assay Principle

The ADVIA Centaur HAV Total assay is a fully automated, competitive immunoassay using direct, chemiluminescent technology. The assay consists of three reagent addition and incubation steps. First, the sample is pretreated with Ancillary Reagent containing cysteine. Next, HAV antigen is added from the ancillary well (Antigen Reagent). Lite Reagent and Solid Phase are then added. The Lite Reagent contains monoclonal mouse antibody to HAV antigen labeled with acridinium ester and biotinylated Fab fragment of a monoclonal mouse antibody to HAV antigen. The Solid Phase contains streptavidin covalently coupled to paramagnetic particles. After the final incubation, the immuno-complex formed is washed with

Wash 1 prior to initiation of the chemiluminescent reaction.

The system automatically performs the following steps:

- dispenses 20 µL of sample and 50 µL of Ancillary Reagent into a cuvette and incubates for 5.75 minutes at 37°C
- dispenses 100 µL of Antigen Reagent and incubates for 28.75 minutes at 37°C
- dispenses 100 µL of Lite Reagent and 175 µL of Solid Phase and incubates for 18.0 minutes at 37°C
- separates, aspirates, and washes the cuvettes with Wash 1
- dispenses 300 µL each of Acid Reagent and Base Reagent to initiate the chemiluminescent reaction
- reports results according to the selected option, as described in the system operating instructions or in the online help system

The relative light units (RLUs) detected by the ADVIA Centaur System are used to calculate the Index Value from the Master Curve. Assay results above the cutoff of the assay are not indicative of antibody level.

Specimen Collection and Handling

Serum, potassium EDTA plasma, and sodium or lithium heparinized plasma are the recommended sample types for this assay. Do not use specimens with obvious microbial contamination. The performance of the ADVIA Centaur HAV Total assay has not been established with cord blood, neonatal specimens, cadaver specimens, heat-inactivated specimens, or body fluids other than serum or plasma such as saliva, urine, amniotic fluid, or pleural fluid.

The following general recommendations for handling and storing blood samples are furnished by the National Committee for Clinical Laboratory Standards,[7] and augmented with additional sample handling studies using the ADVIA Centaur HAV Total assay:

- Handle all samples as if capable of transmitting disease.
- Samples are processed by centrifugation, typically followed by physical separation of the serum or plasma from the red cells. The centrifugation step may occur up to 24 hours post draw.
- Test samples as soon as possible after collecting. Store samples at 2 to 8°C if not tested within 12 hours of collection.
- Store samples in secondary tubes, stoppered and upright at all times at 2 to 8°C up to 7 days.
- Store primary tube samples at 2 to 8°C up to 24 hours. Keep samples stoppered and upright at all times. Primary tube samples include serum stored on the clot, plasma stored on packed red cells, and samples processed and stored in gel barrier blood collection tubes.
- Freeze samples, devoid of red blood cells, at or below -20°C for longer storage. Samples may be stored at or below -20°C for up to 365 days. Do not store in a frost-free freezer. When specimens are subjected to up to 4 freeze/thaw cycles, no clinically significant differences were observed. Thoroughly mix thawed samples and centrifuge at 10,000 g for 2 minutes before using. Collect the supernatant into a clean vial.
- Package and label samples for shipment in compliance with applicable federal and

international regulations covering the transport of clinical samples and etiological agents. Store samples stoppered and upright at 2 to 8°C upon arrival. If shipment is expected to exceed 2 days, ship specimens frozen.

Before placing samples on the system, ensure the following:

- Samples are free of fibrin or other particulate matter. Remove particulates by centrifugation. (example: 1500xg for 10 minutes; follow tube manufacturer's recommendations).
- Samples are free of bubbles or foam.

Reagents

Store the reagents upright at 2–8°C.
Mix all primary reagent packs by hand before loading them onto the system. Visually inspect the bottom of the reagent pack to ensure that all particles are dispersed and resuspended. For detailed information about preparing the reagents for use, refer to Appendix C, Handling Reagents.

Protect from sunlight
Protect reagent packs from all light sources. Reagent packs loaded on the system are protected from light. Store unused reagent packs at 2–8°C away from light sources.

Reagent Pack	Reagent	Volume	Ingredients	Storage	Stability
ADVIA Centaur HAVT ReadyPack primary reagent pack	Lite Reagent	10.0 mL/ reagent pack	monoclonal mouse anti-HAV antibody[1] (~1.0 µg/mL) labeled with acridinium ester and biotinylated monoclonal mouse anti-HAV Fab fragment (~0.08 µg/mL) in phosphate buffer with bovine serum albumin, sodium azide (< 0.1%) and preservatives	2-8°C	until the expiration date on the pack label. For onboard stability, refer to *Onboard Stability and Calibration Interval.*
	Solid Phase	17.5 mL/ reagent pack	streptavidin coupled to paramagnetic particles in phosphate buffer with bovine serum albumin, sodium azide (< 0.1%) and preservatives	2-8°C	until the expiration date on the pack label. For onboard stability, refer to *Onboard Stability and Calibration Interval.*
	Antigen Reagent	10.0 mL/ reagent pack	HAV antigen[2] (~0.06 µg/mL) in tricine buffer with bovine serum albumin, stabilizers, sodium azide (< 0.1%) and preservatives.	2-8°C	until the expiration date on the pack label. For onboard stability, refer to *Onboard Stability and Calibration Interval.*
ADVIA Centaur HAVT AMC ReadyPack ancillary reagent pack	Ancillary Reagent	25.0 mL/ reagent pack	cysteine in citrate buffer with EDTA and preservatives	2-8°C	until the expiration date on the pack label. For onboard stability, refer to *Onboard Stability and Calibration Interval.*
HAVT calibrator vials	Calibrators	2.0 mL/ vial	processed human plasma positive for anti-HAV antibodies with sodium azide (< 0.1%)	2-8°C	until the expiration date on the vial, 60 days after opening vial, or onboard 8 hours
HAVT quality control material vials[3]	Controls	7.0 mL/ vial	processed human plasma negative and positive for anti-HAV antibodies with sodium azide (< 0.1%)	2-8°C	until the expiration date on the vial, or 60 days after opening vial, or onboard 8 hours

ADVIA Centaur APW 1	Probe Wash	25 mL/ pack	0.4 N sodium hydroxide	2-8°C	until the expiration date on the pack label or 14 consecutive days after accessing the ancillary reagent pack
ReadyPack ancillary reagent pack[3]					
ADVIA Centaur WASH 1 [3]	Wash 1	1500 mL/ pack	phosphate buffered saline with sodium azide (< 0.1%) and surfactant	2-25°C	until the expiration date on the vial or onboard 14 days

1 The antibody recognizes a conformational epitope on the assembled hepatitis A virus.

2 HAV Antigen is an inactivated partially purified hepatitis A virus preparation

3 See Materials Required But Not Provided

Precautions And Warnings

For *In Vitro* Diagnostic Use

CAUTION: Sodium azide can react with copper and lead plumbing to form explosive metal azides. On disposal, flush reagents with a large volume of water to prevent the buildup of azides, if disposal into a drain is in compliance with federal, state, and local requirements.

CAUTION! POTENTIAL BIOHAZARD: Some components of this product contain human source material. No known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. All products manufactured using human source material should be handled as potentially infectious. Handle this product according to established good laboratory practices and universal precautions.[8-10]

The calibrators and controls have been assayed by FDA-approved methods and found nonreactive for hepatitis B surface antigen (HBsAg), antibody to hepatitis C (HCV) and antibody to HIV 1/2 and HIV antigen.

The positive control and calibrators contain human plasma that is reactive for anti-HAV total but negative for anti-HAV IgM.

Loading Reagents

Ensure that the system has sufficient primary and ancillary reagent packs. For detailed information about preparing the system, refer to the system operating instructions or to the online help system.

CAUTION: Mix all primary reagent packs by hand before loading them onto the system. Visually inspect the bottom of the reagent pack to ensure that all particles are dispersed and resuspended. For detailed information about preparing the reagents for use, refer to Appendix C, *Handling Reagents*, in the ADVIA Centaur Assay Manual.

Load the ReadyPack primary reagent packs in the primary reagent compartment using the arrows on the packs as a placement guide. The system automatically mixes the primary reagent packs to maintain homogeneous suspension of the reagents. Load the ReadyPack ancillary reagent packs in the ancillary reagent entry. For detailed information about loading reagents, refer to the system operating instructions or to the online help system.

CAUTION: The Low and High Calibrators provided in this kit are matched to the ReadyPack primary reagent pack. Do not mix calibrator lots with different lots of reagent packs.

CAUTION: The Ancillary Reagent provided in this kit is matched to the Lite Reagent, Solid Phase, and Antigen Reagent contained in the primary reagent pack. Do not mix Ancillary Reagent lots with different lots of Lite Reagent, Solid Phase, and Antigen Reagent.

NOTE: The Ancillary Reagent pack contains more volume than required to perform 100 tests. Since the Ancillary Reagent is matched to the Lite Reagent, Solid Phase, and Antigen Reagent in the ReadyPack primary reagent pack, discard the Ancillary Reagent pack when the ReadyPack primary reagent pack is discarded. Do not use beyond the onboard stability.

Onboard Stability and Calibration Interval

Onboard Stability	Calibration Interval
41 days	14 days

Additionally, the ADVIA Centaur HAV Total assay requires a two-point calibration:

- when changing lot numbers of primary reagent packs
- when replacing system components
- when quality control results are repeatedly out of range

CAUTION:

- Discard reagent packs at the end of the onboard stability interval.
- Do not use reagents beyond the expiration date.

Master Curve Calibration

The ADVIA Centaur HAV Total assay requires a Master Curve calibration when using a new lot number of Lite Reagent, Solid Phase, and Antigen Reagent. For each new lot number of Lite Reagent, Solid Phase, and Antigen Reagent use the barcode reader or keyboard to enter the Master Curve values on the system. The Master Curve card contains the Master Curve values. For detailed information about entering calibration values, refer to the system operating instructions or to the online help system.

Calibration

For calibration of the ADVIA Centaur HAV Total assay, use ADVIA Centaur HAV Total Calibrators provided with each kit. The calibrators provided in this kit are matched to the ReadyPack primary reagent pack.

Using Barcode Labels

NOTE: Calibrator barcode labels are lot number specific. Do not use barcode labels from one lot of calibrators with any other lot of calibrators.

Use the ADVIA Centaur HAV Total Calibrator barcode labels to identify the Low and High Calibrator sample cups when performing the ADVIA Centaur HAV Total assay. Place the barcode label on the sample cup so that the readable characters on the side of the label are vertical on the sample cup.

Performing a Calibration

Each lot of calibrators contains a Calibrator Assigned Value card to facilitate entering the calibration values on the system. Enter the values using the barcode scanner or the keyboard. For detailed information about entering calibrator values, refer to the system operating instructions or to the online help system.

NOTE: This procedure uses calibrator volumes sufficient to measure each calibrator in duplicate.

1. Schedule the calibrators to the worklist.
2. Label two sample cups with calibrator barcode labels: one for the low and another for the high.

NOTE: Each drop from the calibrator vial is approximately 50 µL.

3. Gently mix the Low and High Calibrators and dispense at least 4 to 5 drops into the appropriate sample cups.
4. Load the sample cups in a rack.
5. Place the rack in the sample entry queue.
6. Ensure that the assay reagents are loaded.
7. Start the entry queue, if required.

NOTE: Dispose of any calibrator remaining in the sample cups after 8 hours. Do not refill sample cups when the contents are depleted; if required, dispense fresh calibrators.

Quality Control

For quality control of the ADVIA Centaur HAV Total assay, use ADVIA Centaur HAV Total quality control materials. Refer to the Expected Value card for the suggested expected values specific for the lot number of the positive and negative controls. Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

NOTE: The quality control material furnished is intended to monitor substantial reagent failure. If additional controls are desired, it is recommended to run a negative control and a positive control close to the clinically relevant point.

Using Barcode Labels

NOTE: Control barcode labels are lot number specific. Do not use barcode labels from one lot of controls with any other lot of controls.

Use the ADVIA Centaur HAV Total quality control barcode labels to identify the positive and negative sample cups when performing the ADVIA Centaur HAV Total assay. Place the barcode label on the sample cup so that the readable characters on the side of the label are vertical on the sample cup.

Performing Quality Control

For detailed information about entering quality control values, refer to the system operating instructions or to the online help system.

To monitor system performance and chart trends, as a minimum requirement, quality control samples should be assayed on each workshift that samples are analyzed. Quality control samples should also be assayed when performing a two-point calibration. Treat all quality control samples the same as patient samples.

NOTE: This procedure uses control volumes sufficient to measure each control in duplicate.

1. Schedule the quality control samples to the worklist.
2. Label two sample cups with quality control barcode labels: one for the positive, and another for the negative.

NOTE: Each drop from the control vial is approximately 50 µL.

3. Gently mix the quality control materials and dispense at least 5 to 6 drops into the appropriate sample cups.
4. Load the sample cups in a rack.
5. Place the rack in the sample entry queue.
6. Ensure that the assay reagents are loaded.
7. Start the entry queue, if required.

NOTE: Dispose of any quality control materials remaining in the sample cups after 8 hours. Do not refill sample cups when the contents are depleted; if required, dispense fresh quality control materials.

Taking Corrective Action

If the quality control results do not fall within the suggested Expected Values or within the laboratory's established values, then do the following:

- consider the sample results invalid and repeat testing if controls are out of range.
- investigate and determine the cause for the unacceptable control results.
- review these instructions to ensure that the assay was performed according to the procedures recommended by Bayer HealthCare.
- verify that the materials are not expired.
- verify that required maintenance was performed.
- if necessary contact Bayer HealthCare for more assistance.
- when the condition is corrected, retest the controls and confirm that results are within acceptable limits.
- it is advisable to repeat some or all patient specimens before reporting results for this run.

Sample Volume

This assay requires 20 µL of sample for a single determination. This volume does not include the unusable volume in the sample container or the additional volume required when performing duplicates or other tests on the same sample. For detailed information about determining the minimum required volume, refer to *Sample Volume Requirements* in the *ADVIA Centaur Reference Manual.*

Assay Procedure

For detailed procedural information, refer to the system operating instructions or to the online help system.

CAUTION: Do not load more than one size of sample container in each rack. The rack indicator must be positioned at the correct setting for the size of sample container.

1. Prepare the sample container for each sample, and place barcode labels on the sample containers, as required.
2. Load each sample container into a rack, ensuring that the barcode labels are clearly visible.
3. Place the racks in the entry queue.
4. Ensure that the assay reagents are loaded.

5. Start the entry queue, if required.

Procedural Notes

Disposal

Dispose of hazardous or biologically contaminated materials according to the practices of your institution. Discard all materials in a safe and acceptable manner, and in compliance with all federal, state, and local requirements.

Interpretation of Results

For detailed information about how the system calculates results, refer to the system operating instructions or to the online help system.

- The system reports anti-HAV Total results in Index Values.
- Samples with a calculated value of less than 1.00 Index Value are considered nonreactive for antibodies to hepatitis A virus.
- Samples with a calculated value greater than or equal to 1.00 Index Value are considered reactive for antibodies to hepatitis A virus.
- The cutoff for the ADVIA Centaur HAV Total assay was verified based on results of Receiver Operator Characteristics (ROC) Curve and clinical agreement generated from the clinical studies.[11]
- The magnitude of the measured result above the cutoff is not indicative of the total amount of antibody present.
- Sample results are invalid and must be repeated if the controls are out of range.

Limitations

- The ADVIA Centaur HAV Total assay is limited to the detection of total antibodies to hepatitis A virus in human serum or plasma (potassium EDTA plasma, lithium or sodium heparinized plasma).
- The results from this or any other diagnostic kit should be used and interpreted only in the context of the overall clinical picture. A nonreactive test result does not exclude the possibility of exposure to hepatitis A virus.
- The ADVIA Centaur HAV Total assay does not distinguish among different classes of antibodies. The assay cannot be used to determine if a reactive sample is due to an acute infection or is the result of a previous infection. The sample should be tested in a specific HAV IgM assay to determine if there is an ongoing or recent infection.
- The performance of the ADVIA Centaur HAV Total assay has not been established with cord blood, neonatal specimens, cadaver specimens, heat-inactivated specimens, or body fluids other than serum or plasma, such as saliva, urine, amniotic fluid, or pleural fluid.
- The ADVIA Centaur HAV Total assay cutoff is equivalent to 20 mIU/mL standardized to the WHO Second International Reference Standard for Anti-Hepatitis Immunoglobulin (97/646). **However, assay results cannot be considered quantitative and no clinical claims for immunity can be determined from the cutoff.**
- The performance of the assay has not been established for populations of immunocompromised or immunosuppressed patients.

- In patients receiving therapy with high doses of biotin (>5 mg/day) no sample should be taken until at least 8 hours after the last biotin administration.
- Do not use specimens with obvious microbial contamination.
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays.[12] Patients routinely exposed to animals or to animal serum products may develop heterophilic antibodies which may cause interference in immunoassays and, thus, anomalous values may be observed.
- A reactive HAV Total result does not exclude co-infection by another hepatitis virus.

Expected Results

The prospective study population for the ADVIA Centaur HAV Total assay consisted of 846 patients. Of these 846 patients, 249 patients (29.43%) were from the high risk population, 178 patients (21.04%) were from the signs and symptoms population, 2 patients (0.24%) were from the acute HAV infected patient population, 215 patients (25.41%) were from the HAV infected/HAV recovered patient population and 202 patients (23.88%) were from the hospitalized patient population. The prospective study population was 29.20% Caucasian, 37.59% Hispanic, 28.37% Black, 1.65% Asian, and 3.2% from unknown or other ethnicity. The majority of patients were male (58.16% male and 41.84% female). The mean age was 48.42 years (range of 18 to 101 years). Patients in the prospective study population were from the following geographic regions: Florida (58.39%), Texas (29.67%), and New York (11.94%).

The ADVIA Centaur HAV Total results for the prospective population for all sites combined by age group and gender are summarized in the following table.

ADVIA Centaur HAV Total Assay
Distribution of Prospective Population by Age Group and Gender - All Collection Sites

		Reactive (a)		*Non-reactive (b)*		*Total*	
Age (years)	*Gender*	*N*	*%*	*N*	*%*	*N*	*%*
10-19	Female	1	50.00	1	50.00	2	40.00
	Male	1	33.33	2	66.67	3	60.00
	Overall	2	40.00	3	60.00	5	100.00
20-29	Female	16	44.44	20	55.56	36	59.02
	Male	7	28.00	18	72.00	25	40.98
	Overall	23	37.70	38	62.30	61	100.00
30-39	Female	43	60.56	28	39.44	71	47.02
	Male	39	48.75	41	51.25	80	52.98
	Overall	82	54.30	69	45.70	151	100.00
40-49	Female	52	55.91	41	44.09	93	35.63
	Male	102	60.71	66	39.29	168	64.37
	Overall	154	59.00	107	41.00	261	100.00
50-59	Female	56	74.67	19	25.33	75	36.76
	Male	84	65.12	45	34.88	129	63.24
	Overall	140	68.63	64	31.37	204	100.00
60-69	Female	34	75.56	11	24.44	45	45.92
	Male	48	90.57	5	9.43	53	54.08
	Overall	82	83.67	16	16.33	98	100.00
70+	Female	29	90.63	3	9.38	32	48.48
	Male	31	91.18	3	8.82	34	51.52
	Overall	60	90.91	6	9.09	66	100.00
Total	Female	231	65.25	123	34.75	354	41.84
	Male	312	63.41	180	36.58	492	58.16
	Overall	543	64.18	303	35.82	846	100.00

(a) Samples with ≥ 1.00 Index
(b) Samples with < 1.00 Index

As with all *in vitro* diagnostic assays, each laboratory should determine its own reference range(s) for the diagnostic evaluation of patient results.[13]

Performance Characteristics

The prospective study population for the ADVIA Centaur HAV Total assay consisted of 846 patients. Of these 846 patients, 249 patients (29.43%) were from the high risk population, 178 patients (21.04%) were from the signs and symptoms population, 2 patients (0.24%) were from the acute HAV infected patient population, 215 patients (25.41%) were from the HAV infected/HAV recovered patient population and 202 patients (23.88%) were from the hospitalized patient population. The prospective study population was 29.20% Caucasian, 37.59% Hispanic, 28.37% Black, 1.65% Asian, and 3.2% from unknown or other ethnicity. The majority of patients were male (58.16% male and 41.84% female). The mean age was 48.42 years (range of 18 to 101 years). Patients in the prospective study population were from the following geographic regions: Florida (58.39%), Texas (29.67%), and New York (11.94%).

Comparison of Results

The results obtained using the ADVIA Centaur HAV Total assay were evaluated with results obtained using a comparative method for each population category (reactive and nonreactive). The population included 846 prospective subjects and 103 HAV acute retrospective samples. The acute retrospective specimens were characterized as acute if a commercially available anti-HAV IgM test result was reactive. The following results were obtained:

Comparison of Results by Subject Category
ADVIA Centaur HAV Total Assay versus Comparative Anti-HAV Total Assay (All Testing Sites)

	Comparative Anti-HAV Total Assay Negative		*Comparative Anti-HAV Total Assay Positive*		
Subject Category	*ADVIA Centaur HAV Total Assay*		*ADVIA Centaur HAV Total Assay*		
	Reactive	*Nonreactive*	*Reactive*	*Nonreactive*	*Total **
Acute	1	0	104	0	105
High risk	6	125	117	1	249
Signs and symptoms	12	83	83	0	178
Clinical/hospitalized	0	93	109	0	202
Infected/recovered	0	1	214	0	215
Total	19	302	627	1	949

* Total number of test results by Subject Category

Percent Agreement

The percent agreement between the ADVIA Centaur HAV Total assay and the comparative anti-HAV Total assay is summarized in the following table:

Percent Agreement and Confidence Intervals by Subject Category
ADVIA Centaur HAV Total Assay versus Comparative Anti-HAV Total Assay (All Testing Sites)

Subject Category	*Positive Percent Agreement % (x/n)* [1]	*95% Exact Confidence Interval (CI)*	*Negative Percent Agreement % (x/n)* [2]	*95% Exact Confidence Interval (CI)*
Acute	100 (104/104)	96.52 to 100	0 (0/1)	0 to 97.50
High risk	99.15 (117/118)	95.37 to 99.98	95.42 (125/131)	90.30 to 98.30
Signs and symptoms	100 (83/83)	95.65 to 100	87.37 (83/95)	78.97 to 93.30
Clinical/hospitalized	100 (109/109)	96.57 to 100	100 (93/93)	96.11 to 100
Infected/recovered	100 (214/214)	98.29 to 100	100 (1/1)	2.50 to 100
Overall	99.84 (627/628)	99.12 to 100	94.08 (302/321)	90.91 to 96.40

1 x = the number of ADVIA Centaur HAV Total results that are reactive in agreement with the comparative anti-HAV Total results
n = the total number of comparative anti-HAV Total results that are reactive

2 x = the number of ADVIA Centaur HAV Total results that are nonreactive in agreement with the comparative anti-HAV Total results
n = the total number of comparative anti-HAV Total results that are nonreactive

Comparison of Results for Vaccine Recipients

A population of commercially sourced HAV vaccine recipients (with both pre- and post vaccination samples) was tested using the ADVIA Centaur HAV Total assay and a comparative anti-HAV Total assay. All of the vaccine recipients received only the VAQTA vaccine. The following results were obtained:

Comparison of Results in HAV Vaccinated Population
ADVIA Centaur HAV Total Assay versus Comparative Anti-HAV Total Assay (All Testing Sites)

	Comparative Anti-HAV Total Assay Negative		*Comparative Anti-HAV Total Assay Positive*		
HAV Classification	*ADVIA Centaur HAV Total Assay*		*ADVIA Centaur HAV Total Assay*		
	Reactive	*Nonreactive*	*Reactive*	*Nonreactive*	*Total **
Pre-vaccination	0	20	0	0	20
Post- vaccination	1	0	19	0	20
Total	1	20	19	0	40

* Total number of test results by HAV classification

Seroconversion Panels

Commercially available HAV patient seroconversion panels were tested using the ADVIA Centaur HAV Total assay to determine the seroconversion sensitivity of the assay. The performance of the ADVIA Centaur HAV Total assay on the seroconversion panels closely matched the performance of the comparative assay. The following results were obtained:

	Anti-HAV Total Positive Result From Initial Draw Date		Comparative Assay vs ADVIA Centaur Assay
Panel ID	Comparative Assay (Days)	ADVIA Centaur Assay (Days)	Difference in Bleed Numbers *
RP004	7	7	0
RP013	9	9	0
PHT902	16	16	0
ProMedx 1	1	1	0

* The difference in bleed numbers is relative to the comparative assay. In all seroconversion panels both the ADVIA Centaur assay and the comparative assay detected the first reactive sample at the same day

Precision

Precision was evaluated according to the National Committee for Clinical Laboratory Standards protocol EP5-A.[14] Panels of different sample types with five samples in each panel were assayed. Samples were assayed 40 times, with 3 replicates each time, over 17 days, on 1 system. The total CV had a range of 3.3 to 8.0%. The within-run CV was under 4% for all samples. The following results were obtained, using one reagent lot:

	Mean	Within run		Between Run		Total	
Sample	Index Value	SD	CV(%)	SD	CV(%)	SD	CV(%)
Negative Control	0.21	0.03	NA*	0.08	NA	0.10	NA
Positive Control	2.01	0.04	2.0	0.06	3.0	0.10	4.7
K2 EDTA 1	0.47	0.04	NA	0.06	NA	0.09	NA
K2 EDTA 2	1.12	0.04	3.6	0.05	4.5	0.09	7.6
K2 EDTA 3	1.57	0.04	2.2	0.08	4.8	0.10	6.1
K2 EDTA 4	1.82	0.04	2.2	0.07	3.6	0.09	5.0
K2 EDTA 5	2.55	0.04	1.6	0.08	2.9	0.10	3.7
Lithium Heparin 1	0.27	0.04	NA	0.05	NA	0.08	NA
Lithium Heparin 2	1.51	0.04	2.3	0.07	4.3	0.09	6.0
Lithium Heparin 3	1.60	0.04	2.2	0.07	4.4	0.09	5.3
Lithium Heparin 4	1.75	0.04	2.0	0.07	4.0	0.09	4.9
Lithium Heparin 5	2.49	0.04	1.6	0.08	3.2	0.11	4.2
Sodium Heparin 1	0.42	0.04	NA	0.06	NA	0.08	NA
Sodium Heparin 2	1.11	0.04	3.6	0.07	5.9	0.08	7.2
Sodium Heparin 3	1.68	0.06	3.3	0.11	6.3	0.13	7.4
Sodium Heparin 4	1.93	0.04	2.1	0.13	6.8	0.14	7.3
Sodium Heparin 5	2.44	0.04	1.6	0.12	4.9	0.14	5.7
Serum 1	0.37	0.04	NA	0.05	NA	0.10	NA
Serum 2	0.94	0.03	3.2	0.05	4.8	0.08	8.0
Serum 3	1.51	0.04	2.7	0.04	2.7	0.08	5.3
Serum 4	1.71	0.04	2.3	0.06	3.2	0.08	4.7
Serum 5	2.43	0.04	1.6	0.05	1.9	0.08	3.3

* NA = Not applicable

System Reproducibility

The ADVIA Centaur HAV Total reproducibility study was performed at 3 testing sites utilizing 3 reagent lots per site. A 20-member panel, controls, and calibrators were assayed in replicates of 5, on a single run per day, over 6 days, for each lot. Matrices were spiked to targeted Index Value levels and are not matched donors. The study was completed with a single calibration of the assay (one calibration interval). Standard deviation and percent CV were calculated for within-run, between-day, and total.

	Mean Index Value	Within run [1]		Between Run [2]		Between Site [3]		Between Lot [4]		Total [5]		
Sample		SD	CV(%)	SD	CV(%)	SD	CV(%)	SD	CV(%)	SD	CV(%)	N
1-EDTA	0.47	0.05	NA[6]	0.06	NA	0.06	NA	0.00	NA	0.10	NA	270
1-Li Heparin	0.28	0.05	NA	0.05	NA	0.03	NA	0.03	NA	0.09	NA	260
1-Na Heparin	0.44	0.06	NA	0.06	NA	0.07	NA	0.00	NA	0.11	NA	270
1-Serum	0.36	0.06	NA	0.04	NA	0.04	NA	0.00	NA	0.08	NA	270
2-EDTA	1.08	0.05	5.0	0.04	3.6	0.04	3.6	0.03	2.8	0.08	7.7	270
2-Li Heparin	1.50	0.05	3.1	0.05	3.3	0.04	2.6	0.04	3.0	0.09	6.0	270
2-Na Heparin	1.10	0.05	4.4	0.04	3.9	0.03	2.5	0.04	3.4	0.08	7.2	270
2-Serum	0.90	0.05	5.2	0.04	4.8	0.04	4.3	0.01	1.5	0.08	8.4	270
3-EDTA	1.52	0.05	3.1	0.05	3.0	0.03	2.1	0.05	3.1	0.09	5.7	270
3-Li Heparin	1.57	0.05	3.1	0.06	3.8	0.05	3.0	0.05	3.1	0.10	6.6	270
3-Na Heparin	1.68	0.06	3.4	0.05	2.7	0.04	2.6	0.04	2.4	0.09	5.6	270
3-Serum	1.45	0.05	3.5	0.05	3.5	0.04	3.0	0.05	3.6	0.10	6.8	270
4-EDTA	1.79	0.05	2.9	0.04	2.4	0.05	2.8	0.05	2.5	0.10	5.3	270
4-Li Heparin	1.73	0.05	2.8	0.05	3.0	0.04	2.2	0.05	2.8	0.09	5.4	270
4-Na Heparin	1.92	0.06	2.9	0.05	2.5	0.04	1.9	0.07	3.8	0.11	5.7	270
4-Serum	1.67	0.06	3.7	0.06	3.4	0.02	1.3	0.06	3.7	0.11	6.4	270
5-EDTA	2.52	0.06	2.4	0.04	1.6	0.03	1.4	0.07	2.6	0.10	4.1	270
5-Li Heparin	2.46	0.06	2.5	0.06	2.3	0.05	1.9	0.07	2.7	0.12	4.7	270
5-Na Heparin	2.42	0.06	2.5	0.05	2.0	0.04	1.5	0.07	3.0	0.11	4.7	270
5-Serum	2.36	0.06	2.5	0.05	2.2	0.04	1.7	0.06	2.5	0.11	4.5	270
Low Control	0.18	0.08	NA	0.05	NA	0.08	NA	0.00	NA	0.13	NA	270[7]
High Control	2.07	0.06	3.1	0.06	2.7	0.10	4.6	0.00	0.0	0.13	6.2	270

1 Variability of Index Values within day (all testing sites and reagent lots)

2 Variability of Index Values between days (all testing sites and reagent lots)

3 Variability of Index Values between sites (from testing site to testing site, across all reagent lots)

4 Variability of Index Values between reagent lots (from reagent lot to reagent lot, across all testing sites)

5 Variability of Index Values combining (root sum of squares) all four components

6 NA = Not applicable

7 Sixty Low Control results were below the assay reportable range. These results were not used for calculations.

Cross-Reactivity

The ADVIA Centaur HAV Total assay was evaluated for potential cross-reactivity with viral antibodies and disease state specimens. The anti-HAV Total status of each specimen was verified using a comparative anti-HAV Total assay. All specimens that were positive by the ADVIA Centaur HAV Total assay were also positive by the comparative anti-HAV Total assay. The following results were obtained using the ADVIA Centaur HAV Total assay.

		Number of Positive Anti-HAV Total Results	
Clinical Category	**Number Tested**	**ADVIA Centaur Assay**	**Comparative Assay**
Hepatitis C Infection (HCV)	10	4	4
Hepatitis B Infection (HBV)	8	2	2
Rheumatoid Arthritis	9	6	6
Systemic Lupus	2	1	1
Epstein-Barr Virus (EBV) IgG	10	3	3
Epstein-Barr Virus (EBV) IgM	10	3	3
Herpes Simplex Virus (HSV) IgG	10	5	5
Herpes Simplex Virus (HSV) IgM	10	3	3
Cytomegalovirus IgG	10	5	5
Toxoplasma IgG	10	2	2
Toxoplasma IgM	7	3	3
Human Immunodeficiency Virus (HIV1/2)	10	2	2
Varicella Zoster IgG	10	2	2
Rubeola IgG	10	2	2
Anti-Nuclear Antibody (ANA)	5	0	0
HAMA	10	1	1
Flu vaccine Recipient	10	6	6
Total Samples Tested	151	50	50

Endogenous Interferents

The potentially interfering effects of hemoglobin, triglycerides, conjugated bilirubin, unconjugated bilirubin, high protein, low protein, and high human IgG were evaluated. Interference testing was determined according to NCCLS Document EP7-A.[15] In addition, a potential interfering effect of biotin was evaluated using 6 plasma samples spiked with several levels of biotin.

Serum specimens that are . . .	Demonstrate ≤ 10% change in results up to . . .
icteric	* 60 mg/dL of conjugated bilirubin
icteric	* 40 mg/dL of unconjugated bilirubin
lipemic	* 3000 mg/dL of triglycerides
hemolyzed	* 500 mg/dL of hemoglobin
proteinemic	* 3.5 g/dL of protein
proteinemic	* 12.0 g/dL of protein
hyper IgG	* 60 mg/mL of immunoglobulin G
Biotin spiked	* 25 ng/mL of biotin

Technical Assistance

For customer support, please contact your local technical support provider or distributor.

References

1. Hollinger FB, Ticehurst JR. Hepatitis A virus. In: Fields BN, Knipe DM, Howley PM, Fields virology, 3rd ed. Philadelphia, PA: Lippincott-Raven Publishers, 1996:735–782.Stapleton JT. Hepatitis A virus: biology, pathogenesis, epidemiology, clinical description, and diagnosis. In: Specter S, Viral hepatitis: diagnosis, therapy, and prevention; Totowa, NJ: Humana Press Inc., 1999:7–33.
2. Cuthbert JA. Hepatitis A: old and new. Clin Microbiol Rev 2001;14(1):38–58.
3. Bower WA, Nainan OV, Han X, Margolis HS. Duration of viremia in hepatitis A virus infection. J Infect Dis 2000;182:12–17.
4. Siegl G, Lemon SM. Recent advances in hepatitis A vaccine development. Virus Res 1990:17:75-92.
5. Maiwald H, Jilg W, Bock HL, Loscher T, Sonnenburg F. Long-term persistence of anti-HAV antibodies following active immunization with hepatitis A vaccine. Vaccine 1997:15:346-348.
6. Recommendations of the of the Advisory Committee on Immunization Practices (ACIP). Prevention of hepatitis A through active or passive immunization. MMWR 1996:45:(RR-15).
7. National Committee for Clinical Laboratory Standards. Procedures for handling and processing of blood specimens; approved guideline. NCCLS Document H18-A2. Wayne (PA): NCCLS; 1999 Oct. 40p.
8. Centers for Disease Control. Update: Universal precautions for prevention of transmission of human immunodeficiency virus, hepatitis B virus and other bloodborne pathogens in healthcare settings. MMWR 1988;37:377-82, 387-8.
9. National Committee for Clinical Laboratory Standards. Protection of laboratory workers from instrument biohazards and infectious disease transmitted by blood, body fluids, and tissue; approved guideline. NCCLS Document M29-A2. Wayne (PA):NCCLS; 2001 Dec. 105p.
10. Federal Occupational Safety and Health Administration, Bloodborne Pathogens Standard, 29 CFR 1910.1030.
11. National Committee for Clinical Laboratory Standards. Assessment of the clinical accuracy of laboratory tests using Receiver Operator Characteristics (ROC) plots; approved guideline. NCCLS Document GP-10A. Wayne (PA): NCCLS;1995.
12. Boscato LM, Stuart MC. Heterophilic antibodies: a problem for all immunoassays. Clin Chem 1988;34:27–33.
13. National Committee for Clinical Laboratory Standards. How to define, determine, and utilize reference intervals in the clinical laboratory; approved guideline. NCCLS Document C28-A. Wayne (PA): NCCLS;1995.
14. National Committee for Clinical Laboratory Standards. Evaluation of precision performance of clinical chemistry devices–second edition; approved guideline. NCCLS Document EP5-A. Wayne (PA):NCCLS;1999.
15. National Committee for Clinical Laboratory Standards. Interference Testing in Clinical Chemistry; Approved Guideline. NCCLS document EP7-A. NCCLS;vol 22, no 27, 2002.

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